Physiological Degeneration and Death in Entamœba ranarum.

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With 5 Text-figures.

In the following pages, I wish to call attention to some remarkable events which I have observed in the life-history of Entamœba ranarum Grassi, an organism which I have been studying for some time, and whose life-cycle—so far as I had succeeded in following it—I have already described in a previous paper (**2**). I will divide my remarks into two sections —I, a description of the phenomena actually observed, and II, a discussion of their significance.

I.

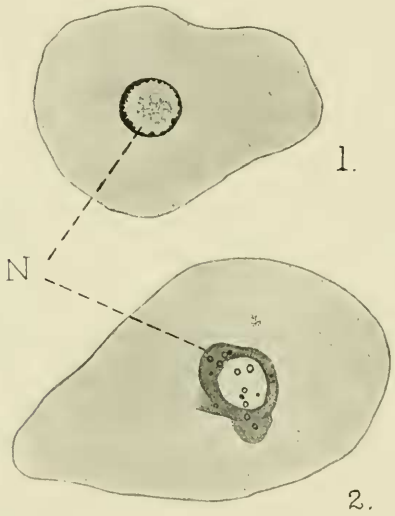
I have given elsewhere (**2**) a detailed description of E. ranarum, but I must here briefly refer to the structure of an ordinary individual once more. A typical amœba, taken from the rectum of a frog or toad, measures—roughly speaking—20–30 μ in diameter, and has a nucleus whose diameter is about 6 μ. For comparison with the forms I am about to describe, I have given a figure of an ordinary organism (text-fig. A, 1), showing the structure of the nucleus. The latter has most of its chromatin in the form of granules arranged peripherally, so that it has an annular appearance in optical section.

Now I occasionally found forms which differ from these

ordinary forms in a most striking manner. They are usually of larger size (40–50μ) and possess a much modified nucleus (text-fig. A, 2). Although this modification varies—both as regards its type and extent—in different individuals, it is nevertheless always characterised by two features—enlargement and peripheral thickening (c f. text-fig. A, 2). Very often, the edge of the nucleus is thrown into folds (text-figs. B, C, 1).

As will readily be seen from the figures, these modified

Text-fig. A.



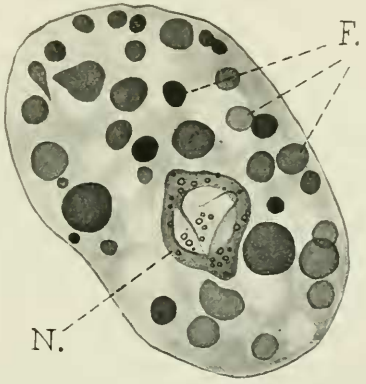
Entamœba ranarum. Drawings (to scale) showing the structure of the nucleus (N) in an ordinary individual (1) and in one undergoing degeneration (2), in optical section. (The structure of the cytoplasm is not shown.)

forms are very striking. For some time I was unable to determine their origin, and their proper place in the life-cycle of the amœba; but I have now been able to prove that these unusual forms are undergoing a process of degeneration, which finally results in death. At first I obtained various isolated stages in the process in animals from different hosts, at different times, but I have now succeeded in following out

the whole process in the amœbæ from two toads which I recently examined (January, 1909; both animals were from the same locality).

The first stage in the process of degeneration consists in an increase in the size of the nucleus, with a well-marked peripheral thickening. All stages intermediate between those shown in text-fig. A, 1 and text-fig. B were to be found. During this process, there does not appear to be any considerable increase in the actual amount of chromatin present in the nucleus: for though the degenerate nucleus often attains twice

TEXT-FIG. B.



E. ranarum, an individual in process of degeneration. N = Nucleus F = Food bodies.

the diameter of the normal nucleus, it nevertheless stains much less deeply with chromatin stains (compare text-figs. A, 1 and 2). The central part is usually almost free from chromatin in the degenerating animals (see text-fig. C, 1). During these morphological changes in the nucleus, chemical changes also take place. A number of refractive granules make their appearance in the nucleus (text-fig. A, 2, etc.). These granules do not take up the nuclear stain, and are very distinct in the living animal. In later stages of degeneration, they are replaced by granules of brown pigment: but whether they are directly converted into pigment, or whether the pigment is a

subsequent formation, I am unable to say with certainty. It appears to me probable that the refractive granules are directly transmuted into pigment. In organisms which have degenerated to a considerable extent, a great deal of pigment may be present in the nucleus (see text-fig. D, 2).

As degeneration proceeds, the nucleus becomes again modified. It tends to become a more uniformly staining mass (text-fig. C, 2), losing its foldings and distinct peripheral thickening. It seems to be undergoing a process of dissolution at this stage. From the fact that many such nuclei were found lying freely in the gut of the host (together with

Text-fig. C.

1 2.

Degeneration nuclei.

enucleate amœbæ), it seems probable that the nucleus may be bodily ejected from the cell at this period. However, I have not observed this in the living animal.[1]

Certainly, in many cases the nucleus now undergoes absorption—the process proceeding until only the granules of brown pigment are left (text-fig. E, 1). These amœbæ are still quite active, and have a very curious appearance whilst alive.

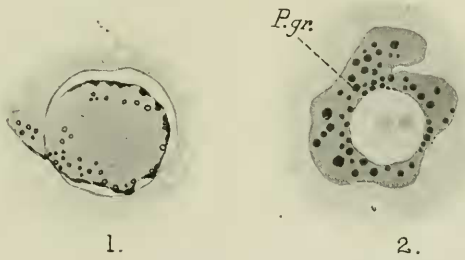
Even the pigment, however, may totally disappear, and amœbæ result which show no vestiges of the nucleus which was originally present (see text-fig. E, 2). These enucleate forms in their last stages, contain practically no food material. It is remarkable that, although in early stages of degeneration the amœbæ are often full of food bodies in various stages of

[1] Prandtl (11) describes this as occurring in Amœba proteus.

digestion (cf. text-fig. B), yet as degeneration proceeds no more food is ingested, and that originally present is but slowly digested. A few undigested remains of food are often seen in the enucleate forms (see text-fig. E, 2), but these are often quite hyaline and free from all trace of cytoplasmic inclusions.

How long these organisms are able to remain alive in this condition when inside their host, I am unable to say. I have observed them undergoing active, and apparently quite normal, movements for many hours under the microscope, before death finally supervened. There is no difficulty in making such observations on the living animals, for—with proper

Text-fig. D.



Degeneration nuclei; in optical section. *P. gr.* = Pigment granules.

procedure—the structure can be observed in the living animal with as much precision as in a fixed and stained preparation.

There is absolutely no evidence to show that the amœbæ are capable of recovery after the processes of degeneration which I have just described have once set in. I should also point out that this kind of degeneration and death differs entirely from that which happens to an ordinary individual when removed from its host. It is also quite different from the simple degenerative changes which sometimes occur in hypertrophied animals which have been kept for some days in cultures of the fæces (see **2**, p. 249).

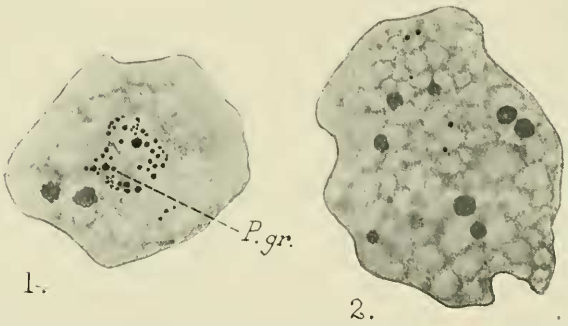
On several occasions I have found degenerate nuclei of the type seen in text-fig. D, 1. In these the whole of the central

mass of nuclear matter has shrunk away from the membrane. I am not sure what takes place subsequently, but it appears probable that such nuclei undergo a simple process of disintegration.

II.

More than thirty years ago, Brandt showed that enucleate fragments of Actinosphærium invariably die. And many subsequent workers—Nussbaum, Gruber, Hofer, Verworn, Balbiani, and others—have proved that this rule is of general

Text-fig. E.



Enucleate amœbæ, in late stages of degeneration. *P. gr.* = Pigment granules.

application. They have proved by direct experiment that if a protozoon be divided into two parts—one containing the nucleus, the other enucleate—then only the nucleate part is capable of carrying out the vital functions of the animal for any length of time. The enucleate part invariably dies—sooner or later. Of especial interest in the present case is the work of Hofer (10). This investigator experimented upon the free-living Amœba proteus. He cut the organism into two parts—one of which contained the nucleus, the other being enucleate. By keeping both parts under constant observation he found that the nucleate part continued to live and perform its various functions in the normal manner. The enucleate

part, on the other hand, though it remained capable of movement for many days, invariably died in the end. Whilst it continued to live, however, it was seen to be incapable of ingesting food material, and apparently had but little power to digest further such food as was already present. Hofer concluded that the power of locomotion exists in the cytoplasm, independent of the nucleus: but that ingestion and digestion of food by the cytoplasm are possible only with the co-operation of the nucleus.

These two important conclusions are supported by the facts which I have just described in Entamœba ranarum. Enucleation in this case, however, is gradual, so that the change of properties in the resulting organism is not so sharply marked as in the case of a vivisected amœba. But the resulting animal, without a nucleus, is—as we have seen—capable of locomotion, but incapable of ingesting and assimilating food.

The phenomena of physiological degeneration and death which I have described in E. ranarum in the preceding section, are paralleled in other Protozoa (cf. Hertwig [**4**, **6**, **8**], etc., Dobell [**1**, **3**]). The most striking parallel is seen in the case of Amœba proteus, which has been shown by Prandtl (**11**) to undergo a process of physiological degeneration very similar to that which I have observed in E. ranarum. I wish here to say a few words regarding the cause and significance of these phenomena. As the majority of the facts have been admirably dealt with already by R. Hertwig, I will limit myself to as few remarks as possible.

Richard Hertwig, who originated the term "physiological degeneration" for these phenomena, seeks to elucidate them by means of his hypothesis of the karyoplasmic relation ("Kernplasmarelationstheorie"). The degeneration is the result of the overgrowth of the nucleus as compared with the cytoplasm. If this overgrowth is not corrected—e. g. by the elimination of nuclear matter—then death results. Hertwig's own observations upon Actinosphærium, Dileptus, Paramecium, etc., speak strongly in favour of this interpretation.

The overgrowth of the nucleus, resulting in a condition of depression or physiological degeneration, may be induced, according to Hertwig, either by over-feeding or by starvation, and also by change of temperature. Let us consider these factors in the case of E. ranarum.

In the first place, it appears to me that neither excess nor deficiency of nutriment can be the cause of physiological degeneration in Entamœba ranarum.[1] I usually found considerable numbers of amœbæ undergoing degeneration together, and among these it was always easy to find all forms from those literally packed with food (cf. fig. B) to those containing practically none. Although the degenerate amœbæ occurred together in large numbers, I cannot believe that an excessive metabolic activity, which accompanied the preceding period of rapid multiplication, could be the cause of degeneration. For I have frequently found perfectly normal individuals present together in equally large numbers.

With regard to temperature, the facts are interesting, though inconclusive. I find on referring to my note-book that the degenerate amœbæ which I have found occurred in January (1908, 1909) and February (1907). Now I believe the development of E. ranarum in the frog normally culminates in encystation. And it is only in the months of December, January, and February that I have ever found encysting animals (see 2). Before encystation, as I have already recorded (2), a very curious process takes place—an elimination of a considerable amount of chromatin from the nucleus. Now, as has already been shown by Hertwig and his school, in many Protozoa the size of the nucleus—as compared with

[1] Prandtl (11) apparently attributed the physiological degeneration which he observed in Amœba proteus to active multiplication, through prolongation of suitable conditions, at a time when multiplication—in the ordinary course of events—would have ceased. The Amœbæ were collected in autumn—"also gegen Schluss der Vermehrungsperiode der meisten freilebenden Protozoen. Indem nun die Tiere durch die gebotenen günstigen Vermehrungsbedingungen zu weiteren Teilungen veranlasst wurden, gingen sie ihrer physiologischen Degeneration entgegen."

the cytoplasm—increases with lowering of temperature.[1] This at once suggests that the events preceding encystation in E. ranarum are somewhat as follows: In winter the lowering of the temperature leads to an acceleration in the growth of the nucleus. In the ordinary course of events the nucleus then regulates its size by extruding a quantity of nuclear matter into the cytoplasm. This act then calls forth in the organism those changes which bring about encystation.

The occurrence of physiological degeneration could thus be explained as follows: When the lowering of temperature occurs, some unknown factor acting upon the amœba prevents the elimination of the excess of nuclear material produced. This, therefore, would lead to considerable increase in the size of the nucleus—which, as I have shown, actually occurs. As the nuclear material is not given up to the cytoplasm the factor which determines encystation does not come into play, so that the animal does not encyst. The excess of nuclear material is gradually turned into pigment, etc., and the unencysted animal finds itself in a highly abnormal condition, from which it is unable to recover. It therefore undergoes degeneration and death.

This gives us, I think, a plausible explanation of the phenomena which I have observed. There yet remains, however, the question—at present unanswerable—What is the factor which, ex hypothesi, prevents the regulation, by elimination of nuclear matter, of the size of the nucleus, and in consequence, prevents encystation and causes death? It is conceivably some toxic body—e. g. a secretion of the host, or the metabolites of the amœbæ themselves, or a toxin produced by the large numbers of bacteria in the host's gut. On the other hand, it may be a factor which lies within the amœba[2]

[1] See, for example, Popoff, "Experimentelle Zellstudien," 'Arch. f. Zellforschung,' Bd. 1, 1908. Lowering of temperature caused both an absolute and a relative increase in the size of the nucleus in the Infusoria studied.

[2] That the overgrowth of the nucleus is itself the primary cause of degeneration and death appears to me highly improbable. I would as soon argue that grey hairs are the cause of old age in man.

—in other words, the organism may lose its power of vital regulation, and so die "naturally."

This last is at least possible. With increase of knowledge it has become necessary to modify considerably the old conception—first precisely stated by Weismann—that the Protista are immortal. We now know that cell-death—complete or partial—is a common phenomenon in the Protozoa. Many of the facts have been already expressed far better than I could express them by Richard Hertwig (see **5**, **9**, etc.). And he concludes: "Im Gegensatz zu Weismann nehme ich an, dass schon im normalen Lebensprocess die Keime des Todes enthalten sind, dass der Tod keine zufällige Anpassung ist, sondern die nothwendige Consequenz des Lebens selbst. Somit können auch die Protozoen nicht unsterblich sein in dem Sinne wie Weismann will; sie würden ebenso zu Grunde gehen müssen wie die vielzelligen Thiere, wenn nicht Einrichtungen getroffen wären, welche die schädlichen Wirkungen des Lebensprocesses compensiren" (**5**, p. 73).

[All the figures are drawn from permanent preparations fixed with sublimate alcohol, and stained with Delafield's hæmatoxylin and eosin. They were all drawn under the Zeiss 2 mm. apochromatic oil imm. (1·40) with compens.-oc. 12.]

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Cambridge,
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